Liquid Collagen Wound Coverings Award Number N00014-90-J1797 Quarterly Report
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INTRODUCTION

Kathleen A. Waldorf, M.D. joined the group in September and was hired as a clinical fellow through the division of Plastic Surgery. Her primary objective is to conduct the clinical trials of the pourable collagen dressing. However, prior to beginning these we had to refine the product somewhat. This included devising a method of sterilization which would satisfy FDA requirements. This, as you know, has been difficult because of various properties of the collagen, including viscosity and radiosensitivity. We feel we have overcome this with an acceptable means of sterilization as I will elaborate on. We also had some concern with the use of iodine in clean wounds as it is felt to impede wound healing. We have arrived at a means of neutralizing the iodine to an inactive form. This gives us the benefit of initially sterilizing the wound bed but without an ongoing impedance of wound healing. With this resolved, we have expanded clinical trials as of December 1, 1990.

COLLAGEN PREPARATION

We have made some changes in the collagen preparation since our last report. These changes are primarily directed at more rigorous aseptic technique. This includes the use of static-hoods and U.V. steri-lamps which have been adapted to the laboratory preparation of collagen. This serves to reduce the physical burden on membrane filtration. In addition, we have added an initial defatting step prior to further processing of the fetal calf skin. This better ensures the production of pure final product of Type I collagen. Defatting of the skin also enables more efficient filtration of the collagen.

An additional concern was the adverse effect iodine may have on wound healing, even though it is used at a very low concentration in our preparation. Iodine has proven to be a very efficient means of cross-linking the collagen and sterilizing the wound bed, making its presence at the initial stages of therapy desirable. However, the persistence of iodine during the fragile re-epithelization process could prove to be detrimental. We devised a means of neutralizing the deleterious effects of iodine by converting it to an inert salt. at various times after the iodocol is applied, buffered ascorbic acid is applied causing the iodine to be reduced to iodide.

STERILIZATION OF COLLAGEN

In spite of rigorous aseptic technique, we found some contamination in our quality assurance cultures. We then proceeded to look at various ways to sterilize the end product so as to make it safe for external and internal application. Gamma irradiation in the amount of 2.5 mega-rads causes fragmentation of the collagen molecule in the absence of DOPA. Similarly, U.V. irradiation, in addition to severe practical limitation (poor penetration), modifies the collagen molecule. Membrane filtration by pressure, carefully used and monitored, appears to be the method of choice. Application specialists in the membrane filter industry have been consulted and a pilot system is being set up. In the meantime,

small scale filtration is being carried out on material for clinical use. We are currently able to filter at a concentration of approximately 5 mg/ml which is appropriate for the pourable wound dressing. We are working on a means of concentrating the collagen, under sterile conditions, after filtration to produce a collagen "putty." The application of this putty will be discussed under the animal studies section.

HUMAN STUDIES

As with previous patients with pressure ulcers and traumatic wounds, the pourable collagen is easily applied and well tolerated. The two patients enlisted since 12/1/90 have both had split thickness skin graft donor site wounds. There is subjectively less pain than experienced at the standard donor site. There has been minimal to no adverse tissue reaction noted. At this point we feel we will enroll 2-3 patients per week and should have a total of 12-15 patients by the end of January. We will continue with donor site wounds initially as they are clean and offer fewer variables. In addition, they are often bilateral which offers "built in" control. We will begin enrolling a variety of wounds as previously described after the initial 10 donor sites are fully evaluated.

ANIMAL STUDIES

In our previously described rabbit ear wound model we have applied the iodocol + ascorbic acid and compared it to iodocol and no therapy in terms of re-epithelization. Our preliminary data suggests no significant different between the two treatments.

GROWTH FACTOR DELIVERY

An interesting new development of this study is the use of our collagen preparation as a delivery vehicle for growth factors. We recently concluded a collaborative agreement with Dr. Jeffrey Hollinger at U.S. Army Institute of Dental Research to study the effect of our collagen preparation on bone healing. We are beginning a pilot study evaluating the efficacy of this collagen "putty," (which is basically very concentrated collagen - 80mg/ml), as a matrix for osteogenin. Osteogenin is a bone inductive factor which is thought to require species-specific collagen to be osteo-inductive. If our collagen proves to be an effective matrix for osteogenin this would, of course, dramatically broaden the clinical application. This study is a collaborative effort of our laboratory, the Division of Plastic Surgery and Dr. Hollinger. The preliminary results are expected in mid-March.

We are currently planning experiments using our collagen as a carrier matrix for additional growth factors for topical and internal application. Preliminary contact has been established with Creative Biomolecules, a group which is making recombinant growth factors, and they have agreed to provide us with growth factors for our experiments.

COVALENT BINDING OF DOPA

A continuing effort of our laboratory is the application of DOPA as a cross-linking agent for collagen. The effect of various conditions of activating DOPA with EDAC and then

inducing the amino group of the lysine residue on collagen to covalently bind to the activated DOPA is being studied. Experiments done with radio-labelled DOPA yielded collagen products with radio-label, indicating the covalent binding of DOPA with collagen. Experiments to date show that an acidic pH is necessary to avoid hydrolysis of the activated DOPA, and that the further reaction of binding collagen to the activated DOPA necessitates an increase in pH. An ultraviolet spectrophotometric method has been standardized to quantify the binding.